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Immunity 8(1): 21-30; Jan 1998

Cytokine 9(11): 913; Nov 1997

J Clinical Inventigation 108(12): 1741-2; Dec 2001

Cell 71(6): 847-56; March 1993

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1752

Identification and Molecular Cloning of a Novel Human TNF Receptor Family Member. V. Saylor, T. Randall, D. Gorman, and A. Zlotnik. DNAX Research Institute, Palo Alto, CA 94304 and the Trudeau Institute, Saranac Lake, NY 12983

The TNF Superfamily includes molecules such as TNFR and FAS and is characterized by pseudorich repeats of cysteine residues in the extracellular region of the molecule. These receptors display a diverse set of functions including cell death, proliferation and immunoregulation while generating very specific activation signals. While performing an EST analysis from a Pro-T (CD44+CD25+CD4-CD8-CD3-) mouse thymocytes, we identified a novel member of this family. We then used the mouse cDNA to isolate its human counterpart, TEASR (T-cell expressed activation specific receptor.) is a 228 a.a. type I transmembrane receptor with distict homolgy to CD40, 41BB and OX-40. Tissue analysis of TEASR shows expression limited to the lymph node, spleen and thymus. CD4 and CD8 cells activated with anti-CD3 and IL-2 show TEASR has a slow increase of expresssion peaking at 40 hours. Antibodies to this receptor confirmed all northern expression data through FACS analysis and western blotting. We are currently in the process of discerning the function of this new molecule. DNAX is supported by Schering-Plough.

1753

NITRIC OXIDE ACTIVATES THE TUMOR NECROSIS FACTOR-α PROMOTER THROUGH AN Sp1-BINDING SITE. S. Wang, W. Wang, R. Wesley, and R.L. Danner (SPON. J. Moss). Critical Care Med. Dept., Clinical Center, NIH, Bethesda, MD 20892)

Nitric oxide (NO) increases tumor necrosis factor (TNF-α) production in differentiated U937 cells by decreasing intracellular cAMP (J Biol Chem 1997; 272(9):5959-5965 and Blood 1997; 90(3):1160-1167). Because cAMP regulates gene expression through both CRE and Sp1 sites, the wild-type (WT) 1311-base pair human TNF-α promoter was compared to CRE (dCRE) or Sp1 (dSp1) deletion mutants for responses to NO and cAMP. Promoters (WT, dCRE, or dSp1) were fused to a tet-responsive transcriptional activator gene (tTA) producing three different pTNF-α/tTA constructs. Each construct was separately co-transfected into U937 cells with a plasmid containing the tet-responsive element and downstream chloramphenicol acetyl transferase reporter gene. NO donors, S-nitroso-N-acetylpenicillamine (SNAP) and S-nitrosoglutathione (SNOG), increased WT TNF-α promoter activity to > 228±41% of basal activity (p≤0.05 for both at 500μM). H89 (30μM), an inhibitor of cAMP-dependent protein kinase, increased WT promoter activity to 167%±9 (p=0.019). Conversely, dibutyryl cAMP (100μM) decreased WT TNF-α promoter activity to 61±5% (p=0.01) of basal activity. The dCRE mutant had decreased basal TNF-α promoter activity (57%±6, p=0.001), but still responded to NO (p=0.045 for SNAP, p=0.084 for SNOG) and cAMP signals (p=0.04 for cAMP, p=0.029 for H89). Conversely, although the dSp1 promoter mutant similarly demonstrated decreased basal activity (52±10%, p=0.003), unlike dCRE, it was completely unresponsive to SNAP (p=0.67), SNOG (p=0.65), and H89 (p=0.84). Furthermore, the addition of Sp1 sites into a heterologous minimal simian virus 40 promoter, converted it from NO unresponsive to responsive in U937 cells. These results suggest that NO can activate the human TNF-α promoter via its Sp1-binding site and that this effect of NO is mediated through decreases in cAMP.

1754

LIGHT, A NEW PRO-APOPTOTIC CYTOKINE MEMBER OF THE TNF SUPERFAMILY, AND LYMPHOTOXIN-α ARE LIGANDS FOR THE HERPESVIRUS ENTRY MEDIATOR (HVEM). D. Mauri[1], R. Ebner[2], R. Montgomery[3], K. Kochel[1], T.C. Cheung[1], G.-L. Yu[2], S. Ruben[2], M. Murphy[2], R.J. Eisenberg[4], G.H. Cohen[4], P.G. Spear[3], and C.F. Ware[1]. [1]La Jolla Institute for Allergy and Immunology, San Diego, CA, 92121 [2]Human Genome Science Inc., Rockville, MD 20850, [3]Northwestern Univ., Chicago, IL 60611, [4]Univ. of Penn., Philadelphia, PA 19104

The Herpesvirus entry mediator (HVEM), a member of the tumor necrosis factor receptor (TNFR) superfamily, allows infection of activated T cells by herpes simplex virus (HSV)-1 and 2 by attachment to the envelope glycoprotein D (gD). Here, we demonstrate that HVEM is a receptor for secreted Lymphotoxin (LT)-α and for LIGHT, a newly discovered cytokine member of the TNF family. We also provide evidence showing that LIGHT, a type-II transmembrane protein of 29-30 kDa, binds to a second receptor of the TNFR superfamily, the LTβR. Furthermore, the direct competition of herpesvirus envelope glycoprotein D (gD) for cellular HVEM-LIGHT binding characterizes gD as a virokine. Finally, we show that LIGHT may be able to block HVEM-mediated herpesvirus infection as well as to induce apoptosis in the adenocarcinoma HT29 cell line. These results suggest a mechanism by which HSV alters immune functions regulated by the HVEM/LIGHT-lymphotoxin system that may allow infection in an immunocompetent host.

1755

A TUMOR NECROSIS FACTOR ALPHA POLYMORPHIC PROMOTER SITE BINDS A B CELL AND MACROPHAGE-SPECIFIC DNA-BINDING PROTEIN. Kathleen E. Sullivan, Mise Oh, and Tushar Dharia. Children's Hospital of Philadelphia, Philadelphia, PA 19104

The gene encoding the polymorphic pro-inflammatory cytokine, TNFα, is located on chromosome 6 within the MHC class III region. We have found that a promoter polymorphism at -308 (-308A) is associated with increased production of TNFα by monocytes and is found with increased frequency in patients with systemic lupus erythematosus. We identified a tissue-specific DNA-binding protein by electrophoretic mobility shift analysis which interacts with the polymorphic promoter site. Significant amounts of this protein were found only in B cells and macrophages. Monocytes do not produce this protein, but upon differentiation into macrophages were able to express it. We examined the affinity of the interaction of this DNA-binding protein for the wild-type promoter sequence versus the polymorphic promoter sequence. Relative affinity was measured by salt titration and cross competition. This DNA-binding protein interacted with the polymorphic sequence (-308A) more avidly than the wildtype sequence (-308G) suggesting that this DNA-binding protein may directly mediate increased transcription of TNFα. Affinity purification of this protein revealed an 85 Kd protein which neither required phosphorylation nor zinc for full activity. The promoter target sequence has no strict homology with that of any known transcription factor. These studies suggest that the TNFα -308A polymorphism may directly mediate increased transcription of TNFα through its interaction with a unique transcription factor.

1756

LYMPH NODE GENESIS IS INDUCED BY SIGNALING THROUGH THE LYMPHOTOXIN BETA RECEPTOR

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The delivery of agonist anti-LTβ-R specific monoclonal antibodies to pregnant LTα-/- mice induced the genesis of lymph nodes in the progeny. This is the first demonstration of the reversal of a genetic defect using an agonist mAb in utero, and shows definitively that signaling through LTβ-R is sufficient to induce lymph node formation. Surprisingly, the lymph nodes which formed included mesenteric, cervical, and sacral nodes, which were thought to develop independently of LTβ-R. Genesis of all lymph nodes was blocked by treatment of normal mice in utero with a combination of LTβ-R-Ig and TNF-R55-Ig fusion proteins, but not with LTβ-R-Ig alone. The data suggest the existence of a novel LTα heterotrimer which can signal membrane-bound LTβ-R in the presence of soluble LTβ-R-Ig fusion protein to induce the formation of mesenteric, cervical, sacral, and lumbar lymph nodes. We have analyzed the phenotype and function of lymph nodes induced to form in LTα-/- mice as compared to those of normal adult mice treated with LTβ-R-Ig or TNF-R55-Ig fusion proteins. Our results suggest that both LT and TNF pathways play critical roles in the formation and maintenance of lymph node cellular organization.

1757

Early Expression of TNF Accelerates Diabetes Development in NOD mice

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The inflammatory cytokine, TNF, has been ascribed a series of positive or negative roles in several disease states. In insulin dependent diabetes mellitus, the importance of TNF has been widely debated. For example, in NOD mice, that spontaneously develop diabetes, studies have demonstrated that administration of TNF to adults protects against diabetes development. Similarly, NOD mice expressing TNF in their islets, starting at 7 weeks of age, do not become diabetic. In contrast to this, neonatal injection of TNF into NOD accelerates diabetes progression. We present evidence that expression of TNF at 18 days of gestation in NOD, leads to rapid onset of diabetes, compared to non-transgenic littermates. Accumulating data from these transgenic animals, has suggested that this rapid progression to diabetes may be related to the ability of TNF to induce β-cell apoptosis early on in the life of these animals, ths providing a source of islet antigens to the immune system. Further, we demonstrate that TNF plays a subsequent role in the priming of the CD4 islet-specific T cell repertoire, which helps provide a more efficient priming of the immune system to islet antigens.